

COMMUNICATIONS FROM THE DEPARTMENT OF ANATOMY

UNIVERSITY OF LUND, SWEDEN

No 10 - 1973

GROWTH HORMONE
AND
LONGITUDINAL BONE GROWTH

BY

KARL-GÖRAN THORNGREN

DEPARTMENT OF ANATOMY

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av

KARL-GÖRAN THORNGREN

Med.kand., Bl.

Akademisk avhandling som med vederbörligt tillstånd av medicinska fakulteten vid universitetet i Lund för vinnande av medicine doktorsgrad kommer att offentligen försvaras å Histologiska Institutionens föreläsningssal (nya byggnaden), fredagen den 23 november 1973 kl. 9.15 (precis)

Avhandlingen försvaras på engelska

GROWTH HORMONE AND GROWTH

KARL-GÖRAN WÄNGBERG

Medicine

Akademisk avhandling som med tillstånd af medicinska
fakulteten vid universitetet i Lund för utnämning af doktors-
grad kommer att offentliggöras i medicinska institutionens
föreläsningssal (nya byggnaden) den 23 november 1973
kl. 9.30

Avhandlingens innehåll: ...



From the Departments of Histology and Orthopaedic Surgery,
University of Lund, Lund, Sweden

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BY

KARL-GÖRAN THORNGREN

Lund 1973



To Margareta and my parents.

"When you can measure what you are speaking about
and express it in numbers, you know something about it, ---"

Lord Kelvin.

The present summary is based on the following papers:

- I. HANSSON L.I., MENANDER-SELLMAN K., STENSTRÖM A., and THORNGREN K.-G.: Rate of normal longitudinal bone growth in the rat. *Calcif. Tiss. Res.* 10, 238-251 (1972).
- II. THORNGREN K.-G., HANSSON L.I., MENANDER-SELLMAN K., and STENSTRÖM A.: Effect of hypophysectomy on longitudinal bone growth in the rat. *Calcif. Tiss. Res.* 11, 281-300 (1973).
- III. THORNGREN K.-G., HANSSON L.I., MENANDER-SELLMAN K., and STENSTRÖM A.: Effect of dose and administration period of growth hormone on longitudinal bone growth in the hypophysectomized rat. *Acta endocr. (Kbh.)* 74, 1-23 (1973).
- IV. THORNGREN K.-G. and HANSSON L.I.: Effect of thyroxine and growth hormone on longitudinal bone growth in the hypophysectomized rat. *Acta endocr. (Kbh.)* 74, 24-40 (1973).
- V. THORNGREN K.-G. and HANSSON L.I.: Effect of withdrawal of growth hormone administration on longitudinal bone growth in the hypophysectomized rat. *Acta endocr. (Kbh.)* in press.
- VI. THORNGREN K.-G. and HANSSON L.I.: Cell kinetics and morphology of the growth plate in the normal and hypophysectomized rat. *Calcif. Tiss. Res.* 13, 113-129 (1973).
- VII. THORNGREN K.-G. and HANSSON L.I.: Cell kinetics of the growth plate in hypophysectomized rats treated with growth hormone and thyroxine. *Z. Zellforsch.* 142, 431-442 (1973).
- VIII. THORNGREN K.-G.: Conditions influencing the dose-response effect of growth hormone on longitudinal bone growth in the hypophysectomized rat. *Comm. Dept. Anat. (Lund)* 7, 1-14 (1973).
- IX. THORNGREN K.-G.: Effect of pituitary hormones on longitudinal bone growth in the hypophysectomized rat. *Comm. Dept. Anat. (Lund)* 8, 1-16 (1973).

- X. THORNGREN K.-G.: Effect of administration frequency of growth hormone on longitudinal bone growth in the hypophysectomized rat. Comm. Dept. Anat. (Lund) 9, 1-16 (1973).
- XI. THORNGREN K.-G. and HANSSON L.I.: Bioassay of growth hormone. I. Determination of longitudinal bone growth with tetracycline in hypophysectomized rats. Acta endocr. (Kbh.) in press.
- XII. THORNGREN K.-G. and HANSSON L.I.: Bioassay of growth hormone. II. Determination of longitudinal bone growth with tetracycline in thyroxine-treated hypophysectomized rats. Acta endocr. (Kbh.) in press.
- XIII. THORNGREN K.-G. and HANSSON L.I.: Bioassay of growth hormone and prolactin preparations by determination of longitudinal bone growth with tetracycline. Acta endocr. (Kbh.) in press.

In the following text, these papers are referred to by their Roman numerals.

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This investigation concerns the stimulating effect of hormones, particularly growth hormone, on longitudinal bone growth. The main objective was to obtain more and better information about the stimulation of growth. Effects of different hormone preparations on longitudinal bone growth were determined in the hypophysectomized rat with tetracycline as marker.

Most earlier investigations of the growth process have been descriptive only. The normal growth process and also the effect of experimental interference have thus been described in morphological terms. Quantitative studies have mostly considered the length of the body or its parts, or the length and width of individual bones, determined directly or with the aid of radiographic techniques. Tetracycline as an intravital marker of longitudinal bone growth permits more precise and sensitive determinations of the growth from one particular growth plate (Hansson 1967). This method has not previously been used for thorough studies of hormonal influence on longitudinal bone growth.

Many investigations have been made into the effects of hypophysectomy and hormone administration on the growth plates of the long bones, especially the proximal growth plate of the tibia (see Geschwind and Li 1955, Papkoff and Li 1962, Rieckstniece and Asling 1966, Asling et al. 1965, 1968). The histological changes have been described, and often the width of the growth plate has been determined by using X-ray or stained sections as an indirect index of the growth in length (for review, see papers II and III).

It is well known that hypophysectomy retards growth and that subsequent growth hormone administration results in resumed growth (for review, see Smith 1930, Simpson et al. 1950, Asling and Evans 1956, Sissons 1956, 1971, Evans et al. 1966, Root 1972, Urist 1972). The growth stimulating effect of thyroxine has varied in different earlier investigations (for review, see paper IV). Combined administration to hypophysectomized rats of growth hormone and thyroxine causes more rapid growth than either hormone given alone (for review, see Simpson et al. 1950, Asling and Evans 1956, paper IV).

In the present study the more precise determination by tetracycline of the influence of growth hormone and thyroxine on longitudinal growth from one growth plate of a long bone permitted detailed studies of hormonal regulation of the growth in length. The longitudinal bone growth rate was compared with the morphology of the contralateral growth plate and calculations of cell production were performed.

For bioassay of growth hormone, many different methods have been used (for review, see paper XI). A species specificity of growth hormone exists in many animals, but the rat is sensitive to growth hormone from many other species and has therefore been widely used in the bioassay of growth hormone (see Papkoff and Li 1962, Geschwind 1966, Hayashida 1972).

Different growth parameters have been used as in vivo methods for bioassay (for review, see Greenspan et al. 1950), but the longitudinal bone growth has not been possible to use in a sensitive bioassay method. The body weight increase in normal rats (Evans and Simpson 1931), in dwarf mice (Fønss-Bech 1947), and in hypophysectomized rats (Evans et al. 1938, Marx et al. 1942) were early methods for the bioassay of growth hormone; also recent modifications of the body weight tests have appeared (Goth et al. 1964, Parlow et al. 1965). The effects of growth hormone on the increase in tail length of hypophysectomized rats (Dingemanse et al. 1948, Baume et al. 1958, de Groot 1963) and on the metabolism (for review, see Greenspan et al. 1950, Russel 1955, Papkoff and Li 1962) have also been used. The most widely used bioassay of growth hormone is the tibia test determining cartilage width (Evans et al. 1943, Greenspan et al. 1949, Geschwind and Li 1955, Papkoff and Li 1962, Cargill Thompson and Crean 1963).

In recent years, immunologic assay methods for growth hormone have been developed (for review, see Evans et al. 1966, Hayashida 1966, Root 1972). These are very sensitive compared with available bioassay methods (Loraine and Bell 1966). However, the immunoassay is dependent on the biochemical properties of the substance used for the immunization process. It is the immunologic resemblance of this substance that is determined, not its biologic effects. It is well known that immunologic and biologic determinations can give different results (see Müller et al. 1972).

Growth hormone is prepared by extraction from pituitaries; synthetic growth hormone is not available at present, although its amino acid sequence is known (see paper XI). The bioassay methods have been very important as test procedures for growth hormone activity in the development of isolation- and purification-methods (Evans et al. 1966). They are necessary for testing the biologic activity of various growth hormone preparations or active parts of the growth hormone protein molecule. They are also indispensable for the evaluation of synthetic substances with growth promoting activity.

For clinical substitution therapy, the intended effect of the human growth hormone administration is to stimulate longitudinal growth. However, of the earlier bioassay methods for growth hormone, only few measure the actual longitudinal bone growth.

The present study was aimed at developing a bioassay method for growth hormone with tetracycline as an intravital marker of longitudinal bone growth from the proximal growth plate of the tibia in the hypophysectomized rat, the objective being to allow a more precise quantitative determination of the growth stimulating effect of different growth promoting preparations.

2 MATERIAL AND METHODS

2.1 Rats

Sprague-Dawley rats were used. The rat is convenient to keep and handle on a large scale and is known to be sensitive to growth hormone. In most previous investigations of the influence of growth hormone on longitudinal growth, female rats have been used (Asling and Evans 1956, Evans *et al.* 1966, Urist 1972). In the hormone experiments, the present study too used only female rats to facilitate comparison with earlier investigations.

Sprague-Dawley rats with known date of birth were delivered from the breeding farm about one week before growth determination or hypophysectomy. The sex and the age of the animals, as well as the breeding, have an influence on the normal longitudinal bone growth, as shown in paper I. In the hormone experiments (papers III-V, VII-XIII) these conditions were standardized. Only female rats hypophysectomized at exactly the same age were used. The rats were delivered from the same breeder (Møllegaard, Denmark) in all hormone experiments. They were taken from different litters, the important factor being the exact day of birth, because the use of complete, separate litters increases the animal variation (paper I).

The animals were kept in clear plastic cages with a metal grill top in an air-conditioned room with daylight illumination. The temperature and the relative humidity were standardized. All animals were given the same pellet diet (Anticimex 213) and water ad libitum.

2.2 Hypophysectomy

The rats were hypophysectomized by the parapharyngeal approach (Smith 1930, Jacobsohn 1966). They were anaesthetized with ether and intubated with a glass cannula to prevent obstruction of the airway during the operation. The base of the skull was dissected free, a hole made in its base by a dental drill, and the hypophysis removed by suction with the aid of a bent glass pipette. At the hypophysectomy, some animals were given cortisone acetate (Cortone [®]) subcutaneously (papers II, III, VI-XIII). The rats were hypophysectomized at 60 days of age, except when the effects of operation and hormone administration at different ages were studied as in papers II and III.

The completeness of the hypophysectomy was determined microscopically by serial section and histological examination, as described by Jacobsohn (1960, 1966). A

block of tissue including base of skull, and pituitary capsule and stalk was fixed in Bouin's solution. The dura mater with the capsule and the pituitary stalk attached was dissected free from the bone, dehydrated, embedded in paraffin wax, and cut into serial sections (12 μ). The sections were stained with haematoxylin and eosin (papers II and III) or with azan (papers IV, V, VIII-XIII). The hypophysectomy was considered complete when no adenohypophyseal cells were found, apart from small pars tuberalis cells lining the pituitary stalk (Jacobsohn 1960).

2.3 Hormones

The influence of growth hormone on longitudinal bone growth was studied by the use of bovine standard preparations from the National Institute of Arthritis and Metabolic Diseases, USA. Owing to the limited amount of material received, two different standard preparations of bovine growth hormone were used (NIH-GH-B15 in papers III, V, and VII; NIH-GH-B16 in papers IV, VII-XIII).

L-thyroxine was used in papers IV, VII-XIII.

Cortisone acetate (Cortone [®]) was used in papers II, III, VI-XIII.

In paper IX preparations of different pituitary hormones were used:

Hormones of the anterior pituitary gland:

Thyroid-stimulating hormone – TSH (Actyron [®])

Prolactin (NIH-P-B2)

Luteinizing hormone – LH (NIH-LH-S7)

Follicle stimulating hormone – FSH (NIH-FSH-S2)

Adrenocorticotrophic hormone – ACTH (Acton [®])

Hormone of the pars intermedia:

Melanocyte-stimulating hormone – MSH (α MSH)

Hormones of the posterior pituitary gland:

Vasopressin (Postacton [®])

Oxytocin (Partocon [®])

In paper XIII different growth hormone and prolactin preparations were used:

Bovine growth hormone: WHO (1st International Standard for growth hormone), and NIH-GH-B17

Human growth hormone: HS 1523 D, Crescormon [®], and HGH 59

Ovine growth hormone: NIH-GH-S9

Porcine growth hormone: P 526 B, and Somacton [®]

Bovine prolactin: NIH-P-B2

Ovine prolactin: NIH-P-S5

2.4 Growth region

Earlier investigations have shown that the longitudinal bone growth is different for the different growth plates of a long bone (see Hansson 1967). In the present investigation, the hormonal influence on the longitudinal bone growth was determined in the proximal growth plate of the tibia, which has a very regular growth pattern, facilitating determination of the longitudinal bone growth with tetracycline as intravital marker. This growth region has been widely studied in earlier experiments concerning hormonal influence on the morphology of the growth plate and its width (for review, see Geschwind and Li 1955, Asling and Evans 1956, Sissons 1956, 1971, Papkoff and Li 1962, Evans et al. 1966, Urist 1972) making it possible to compare the present investigation with the earlier ones.

2.5 Determination of longitudinal bone growth

To determine the longitudinal bone growth, the experimental animals were given consecutive doses (10 mg/kg) of oxytetracycline, OTC (Terramycin[®]). As known from earlier investigations, this dose has no visible side effects (see Hansson 1967, Hansson et al. 1968, paper I). The injections were given intraperitoneally, and the rats were temporarily anaesthetized with ether to increase the accuracy of the injections (see paper I).

After the experimental period, the rats were killed with ether. The proximal tibia of the right side was dissected out and the bones were kept in absolute alcohol for about 24-48 hours. Undecalcified sections were produced according to a method described earlier for the rabbit (Hansson 1964, 1967). The procedure was the same, but the sectioning and growth determination was found to be more satisfactory for the medial condyle of the proximal tibia in the rat. The sections were cut manually with a razor blade and immersed in xylol for about 5 min before being mounted in DePeX[®] between glass.

The growth determination was made by the use of a fluorescence microscope according to the principles described by Hansson (1967) with a methodological error of about 5 μ (Hansson 1967, Hansson et al. 1973). Measurements were made between fluorescent bands in the metaphysis corresponding to the injections of OTC. The mean value for each animal was calculated from a total of 10 determinations in 5 sections. In the hormone experiments (papers III-XIII), the measurements were made between the two fluorescent bands

corresponding to the injections of OTC given at hypophysectomy and 15 days postoperatively. Measurements were also made between the second fluorescent band and the erosion line situated between the metaphysis and the growth plate, about 0–20 μ below the zone of endochondral calcification (see paper III).

The width of the fluorescent band corresponding to an injection of OTC is correlated to the growth rate and can be used as an index of the growth rate (see paper II). In the hormone experiments, the width of the fluorescent band corresponding to the injection of OTC 15 days after hypophysectomy was determined as a measure of the effectiveness of hypophysectomy.

2.6 Morphology and cell kinetics of growth plate

The proximal tibia of the left side was dissected out from all the animals. Decalcified longitudinal and frontal sections stained with haematoxylin and eosin were investigated microscopically (Hansson 1967). The total width of the cartilage growth plate and the width of its undifferentiated and columnar zones were determined as described by Hansson (1967). The mean value for each animal was calculated from a total of 10 determinations in 5 sections. The size of the degenerative cell situated close to the metaphysis in the growth plate was determined. For each animal, the mean value was calculated from a total of 25 determinations in 5 sections measured with 16x planachromatic objective and 12.5x ocular equipped with a calibrated micrometer.

The rate of longitudinal bone growth is equal to the product of two factors in the growth plate: the rate of production of cells per column, and the size of the degenerative cell (Sissons 1955, Kember 1971 a, b, Kember and Walker 1971, Walker and Kember 1972 b). In the present study, the cell production in the proximal growth plate of the tibia was calculated from the longitudinal bone growth determined with OTC, by division with the size of the degenerative cell determined in the same animal (papers V–VII).

2.7 Weight of body and heart

The body weight of the animals was registered at different times. In the hormone experiments, it was registered at hypophysectomy, when the OTC injections were given, at the end of the administration period, and when the animals were killed.

The weight of the heart ventricles (heart weight) was registered when the animals had been killed. The atria were dissected free from the heart ventricles, which were kept

in a moist chamber for a short interval before being weighed (Jacobsohn, personal communication).

2.8 Statistical methods

General statistical procedures (according to Fisher 1950 and Bliss 1952) were used for the calculation of mean value, standard deviation, standard error of mean, Student's t-test, and regression analysis.

Corrected longitudinal bone growth. - Corrected values for the longitudinal bone growth were calculated (see paper XI). It was found that animals having a high longitudinal bone growth during the control period 0-15 days postoperatively, also had a high growth in length for various doses of growth hormone during the experimental period. A model for linear adjustment resulted in the lowest standard error of mean at the various dose-levels. The corrected values of longitudinal bone growth were obtained by the linear adjustment:

$$\text{corrected response } y' = y - b (v - \bar{v}),$$

y and v being measured growth during the experimental and the control period, respectively; $\bar{v}$ the mean control growth, and b the regression of experimental on control growth. In 900 animals, $\bar{v}$ was found to average 550 μ (see paper XI). This value was used in all the calculations. The slope (b) used for correction was calculated by covariance analysis (Bliss and Marks 1939) separately in each different injection model. Corrected values for longitudinal bone growth were calculated in papers VIII-XIII.

Dose-response-line. - In the bioassay experiments, the log dose-response-lines were calculated by regression analysis, y being growth response (accumulated longitudinal bone growth in μ) and x being log total dose (μg) of the growth stimulating hormone. The index of precision (λ) was calculated according to Bliss (1952) as the ratio of the standard deviation (s) over the slope (b) of the line.

Potency calculation. - For each hormone preparation, three groups of animals were given geometrically progressing doses (a, 2a and 4a μg). For each hormone, the regression equation for the log dose-response-line was calculated by the method of least squares. The WHO preparation was tested in the same way. Then the growth stimulating effect (corrected longitudinal bone growth) of each hormone was compared with that of the WHO preparation, which by definition contains 1 IU/mg. The potency and the potency range ($P = 0.95$) were calculated. In addition, the validity of the assays was checked with

regard to the parallelism of the log dose-response-lines (Bliss 1952). The potency determinations were based on comparison with a standard curve obtained at different periods. This was done following the observation of constant responses to a standard preparation at different occasions (see papers VIII, X-XIII).

3.1 Longitudinal bone growth in the rat normally and after hypophysectomy (Papers I and II)

In the normal rat, the rate of growth in length from the proximal growth plate of the tibia was determined at the age of 20-100 days (paper I). The growth rate was found to vary only slightly within the different age groups. It was highest in young animals and decreased considerably with increasing age. Also a sex difference was found, male rats growing faster than female. The age and sex of the animals as well as breeding had an influence on the normal growth rate. These conditions should be standardized as much as possible when studying growth rates (paper I).

Tetracycline has previously been used to study the growth process in the rabbit. Since Hansson (1964, 1967) developed a method with tetracycline as intravital marker of the longitudinal bone growth, several investigations concerning experimental influence on growth in length have been performed in the rabbit (Sundén 1967, Persson 1968, Hedström 1969, Christensen 1973). In the rat, tetracycline has mostly been used to study the formation of lamellar bone (for review, see paper I) and dentine (see Ahlgren 1968). Only a few investigations with tetracycline as marker of the longitudinal bone growth have been reported in the rat (see paper I). In the present study (paper I) the tetracycline method has provided a more exact and detailed knowledge of the normal growth in length from one growth plate in the rat, thereby giving a base for the evaluation of experimental influence on the growth in length.

Hypophysectomy was found to have a retarding effect on the longitudinal bone growth (paper II). In animals operated at 40 days of age, the growth did not cease. The longitudinal bone growth was found to decrease postoperatively and reach a low basal level. When hypophysectomy was performed at 30, 40, 50, or 60 days of age, young animals had a higher basal growth rate than older animals. A single high dose of cortisone acetate (45 mg/kg) given at hypophysectomy was found to have a growth retarding effect postoperatively (paper II).

Tetracycline has not previously been used to determine the growth in length of the long bones after hypophysectomy (see paper II). The tetracycline method was used to obtain more precise information of the effect of hypophysectomy on longitudinal bone growth from one growth plate in the rat (paper II). This information is necessary for the evaluation of the influence of different hormones on the growth in length. It is of importance to know

when the maximum growth retarding effect of hypophysectomy is accomplished, and also whether it is influenced by other factors, such as the age of the operated animal or cortisone therapy given at operation. In experiments concerning the effect of postoperative hormonal substitution therapy on the growth in length, it is necessary to know the most suitable time for the administration to begin, i.e. when the animals have reached a low growth rate. This was determined and further discussed in paper II.

3.2 Control of hypophysectomy by determination of longitudinal bone growth (Papers II, III, and IV)

To determine the completeness of a hypophysectomy, different methods have been used, but microscopical examination of serial sections of sella turcica is regarded as the most exact (for review, see Jacobsohn 1966, paper II). This method was used in all hypophysectomized animals of the present study. After hypophysectomy the width of the fluorescent band, corresponding to a postoperative injection of oxytetracycline, was found to be correlated to the growth rate and could be used as an index of the growth rate (paper II). The postoperative accumulated growth in length and the width of the fluorescent band could be used to indicate the functional efficacy of the hypophysectomy, making it possible to separate animals with complete and with incomplete hypophysectomy (papers II-IV). This method of separation was found to be in accordance with the results of the microscopical determination of sella turcica (papers V, VIII-XIII). The new control method is of value in experiments where serial sectioning of the sella turcica is regarded as too laborious, or where the other indicating target organs have been ablated or used as parameters for determination of experimental influence.

3.3 Effect of growth hormone and thyroxine on longitudinal bone growth in the hypophysectomized rat (Papers III, IV, and V)

Growth hormone was found to stimulate the longitudinal bone growth in hypophysectomized rats. The growth stimulation was dependent on the dose and the length of the administration period. The age and cortisone administration at hypophysectomy had an influence on the growth hormone-induced growth stimulation (paper III). When the growth hormone administration was stopped, the longitudinal bone growth continued for some days after withdrawal (paper V).

An antagonism between growth hormone and glucocorticoids in hypophysectomized

rats was found long ago by means of growth hormone and ACTH (Marx et al. 1943). Later, a competitive antagonism by cortisone of the linear growth stimulated by growth hormone has been shown in hypophysectomized rats (Soyka and Crawford 1965). In the present study, a peripheral effect of cortisone with a lasting depression on the growth process was found for a single dose of cortisone given at hypophysectomy, as discussed in paper III. The growth depressing effect of such a single injection has not been shown before. This high dose of cortisone significantly increased the postoperative survival rate (paper III). A considerably lower cortisone dose (0.5 mg/kg) could be used to increase the postoperative survival somewhat, without having any depressing influence on the growth hormone-induced longitudinal bone growth (paper VIII).

In most previous experimental investigations (see Asling and Evans 1956, Evans et al. 1966, Urist 1972) the growth hormone administration in hypophysectomized rats was continued until the end of the experimental period, whereafter the animals were killed. A continued growth effect after withdrawal of the growth hormone administration, as shown in paper V, has not previously been reported. This effect is also impossible to determine if the width of the growth plate or the body weight is used as growth parameter, because these decrease after the withdrawal, as shown in paper V.

In the present study, thyroxine given alone was found to stimulate the longitudinal bone growth up to an optimum dose (paper IV). Thyroxine and growth hormone given in association resulted in a potentiating synergistic growth stimulation. At the optimum thyroxine dose, the dose of growth hormone determined the increase in longitudinal bone growth (paper IV).

Thyroxine participates both in growth and differentiation of bones (Rieckstniece and Asling 1966). However, the effect on growth in length by thyroxine given to hypophysectomized rats has been variable. In some studies, thyroxine has been shown to promote growth in length (see Simpson et al. 1950, Scow 1954, Asling and Evans 1956, de Groot 1963, paper IV); in others, this effect has not been found (see paper IV). In the present study, thyroxine was found to be growth stimulating up to an optimum dose. It was also possible to demonstrate a statistically significant potentiating synergistic growth stimulation by thyroxine on the growth hormone-induced longitudinal bone growth. The synergism is in accordance with earlier investigations (see Asling and Evans 1956, paper IV).

In the present study, the detailed quantitative information obtained in standardized experiments is of value both to clarify the growth regulation by growth hormone and thyroxine, and to compare their effect with the possible growth promotion of various other substances. These investigations can also provide a base for comparison with other experi-

mental influence on the growth process.

3.4 Cell kinetics of the growth plate in the rat (Papers V, VI, and VII)

The decrease in longitudinal bone growth with increasing age and after hypophysectomy was found to be due partly to a decrease in cell production, and partly to a decrease in degenerative cell size in the growth plate. The changes in cell size, however, were minor, so the influence on cell production dominated (paper VI). Also the changes in longitudinal bone growth induced by growth hormone and thyroxine in hypophysectomized rats were found to be caused predominantly by changes in the cell production, whereas the changes in the size of the degenerative cells were minor (papers V and VII).

For a review of the process of endochondral bone growth, see Sissons (1956, 1971), Hansson (1967), Mc Lean and Urist (1968), and Ham (1969). The rate of longitudinal bone growth is equal to the product of two factors in the growth plate: the rate of production of new cells per column and the size of the degenerative cell (see Sissons 1955, Kember 1971 a, b, Kember and Walker 1971, Walker and Kember 1972 b, papers V-VII). In the present study, the longitudinal bone growth and the size of the degenerative cell were determined in the same animal. Thus, it was possible to compare the longitudinal bone growth from a growth plate with the morphology of the contralateral growth plate and to calculate the rate of cartilage cell production. The rate of growth in length is the same from the right and from the corresponding left growth plate in one and the same animal (Hansson 1967).

Tritium labelled thymidine has been used in autoradiographic experiments to study the growth plate in the rat by a number of workers (Kember 1960, 1971 a, b, Rigal 1962, Dixon 1971, Walker and Kember 1972 a, b). In normal and hypophysectomized rats, the calculated cell production by the method used in the present study was found to agree well with the cell production determined with tritiated thymidine (see paper VI). The autoradiographic studies with tritiated thymidine in vivo are rather laborious, as a large number of cells in each animal has to be counted to calculate the mitotic index. Also a comparison with the longitudinal bone growth demands further calculations (Kember 1972 a). The method in the present study is less tedious and is directly linked to the determined values of longitudinal bone growth.

The present study determines the number of degenerative cells that have been destroyed at the erosion line during the process of endochondral bone formation in the metaphysis. Under normal conditions, the number of degenerative cells is identical with the number of mitoses in the zone of proliferation in the growth plate (see paper VI), and as

discussed in paper VII, also the mean cell production after hypophysectomy and subsequent hormonal administration reflects the mean number of mitoses in the proliferation zone of a growth plate. Thus, the determined values for longitudinal bone growth reflect the changes in mitotic activity under different conditions. This means that all the curves showing longitudinal bone growth also can be interpreted as showing the cell production, and it is possible to calculate the cell production, e.g., for the different doses of growth hormone in the bioassay investigations.

3.5 Effect of growth hormone and thyroxine on different growth parameters (Papers I-VI)

Various growth parameters other than longitudinal bone growth in the rat were influenced by hypophysectomy and administration of growth hormone and thyroxine in a similar way, but the changes were less pronounced.

Width of growth plate. - The total width of the growth plate of proximal tibia was found to decrease in normal rats with increasing age and also after hypophysectomy (paper VI). In hypophysectomized rats given growth hormone, the width of the growth plate was higher than in untreated controls. The width increased with the dose, but not with the administration period (paper III). Thyroxine alone did not cause any increase in the width of the growth plate, whereas thyroxine and growth hormone given in association resulted in an increase in the width corresponding to that of growth hormone given alone (paper IV). Within the growth plate, the undifferentiated zone showed only minor changes, whereas the columnar zone changed similar to the total cartilage width.

Body weight. - The body weight increased with the age in the normal rat (paper I). After hypophysectomy, there was a decrease in the body weight to an almost constant value (paper II). The body weight was increased by growth hormone administration in hypophysectomized rats (papers III and V). Thyroxine administered alone resulted in a decrease in the body weight that was compensated by the administration of growth hormone in association with the thyroxine (paper IV).

Heart weight. - The weight of the heart ventricles was found to be directly related to the body weight in hypophysectomized rats given growth hormone (papers III, V). The heart weight was found to increase with increasing doses of thyroxine, both when given alone and in association with growth hormone (paper IV).

The administration of growth hormone and thyroxine induced quantitatively different changes in the various growth parameters determined in the present study. As

shown in paper V, the administration of growth hormone initiated a growth process in all the parameters studied, but the time necessary to reach an optimum effect was found to differ. After withdrawal of the growth hormone administration, the effect on the studied growth parameters also differed, as discussed in paper V.

Thyroxine was found to act as a stimulator of the process involved in endochondral bone growth, with a considerable growth stimulation even if the growth plate was narrow (paper IV). This would indicate that there is no direct correlation between the growth rate and the width of the growth plate under experimental conditions, as has been claimed previously (Rang 1969). Also after hypophysectomy (paper VI), after administration of growth hormone (paper III), and after withdrawal of growth hormone administration in hypophysectomized rats (paper V), a discrepancy was found between the longitudinal bone growth and the width of the growth plate. This is in accordance with Sissons (1956), Hansson (1967), and Kember (1971 a, 1972) who also found that the relation between width of the growth plate and bone growth is not a correlation of cause and effect under experimental conditions. Thus, after experimental influence on the growth process, it seems more accurate to determine the bone growth directly as in the present study, and not to predict it from the width of the growth plate, as has been the custom in previous investigations.

Preliminary observations have also shown that the stimulation by growth hormone is different in various types of calcified tissue. In the rats of the present study, the apposition of dentine and the lamellar bone growth seem to react similar to the longitudinal bone growth after hormonal influence, but to a quantitatively lesser extent.

The body weight and the heart weight changed similarly in the present study, except after administration of thyroxine (papers III-V). Thus, after hypophysectomy and subsequent administration of growth hormone, this double weight determination is merely a means of getting more precise information of the treatment on weight as a growth parameter. The stimulation of the heart weight by thyroxine is probably due to increased work demands on the heart, as discussed in paper IV.

3.6 Bioassay of growth hormone - factors important for determination of the dose-response effect (Papers III-V, VIII-X)

For the bioassay of growth hormone, it is necessary to know the various factors that might influence the growth response for a specific dose of growth hormone. Generally in a bioassay model, the effect of growth hormone should increase with increasing dose and ideally be constant for the different dose-levels. A high dose-response effect is

favourable. In the present study, the following factors important for the dose-response effect were determined:

Growth parameters. - The growth response related to the dose of growth hormone was compared for different growth parameters by the index of precision (Bliss 1952) in three different experimental models (paper III). The accumulated longitudinal bone growth was found to be the best growth parameter rather than the width of the growth plate, the body weight, and the heart weight. This was also confirmed in the following bioassay investigations (papers XI-XIII).

Age and cortisone administration at hypophysectomy. - When the longitudinal bone growth was used as growth parameter, animals hypophysectomized at 60 days of age without cortisone administration were found to be most suitable for determination of the growth stimulation of various doses of growth hormone (paper III). Thus, rats hypophysectomized at this age were used in the following hormone experiments (papers IV, V, VIII-XIII).

Cortisone administration at hypophysectomy significantly increased the postoperative survival, but it also had a depressing influence on the growth hormone-induced longitudinal bone growth as found in paper III. A considerably lower cortisone dose (0.5 mg/kg) was found to increase the postoperative survival somewhat, without having any postoperative depressing influence on the growth hormone-induced longitudinal bone growth (paper VIII). This cortisone dose was therefore used in papers IX-XIII.

Increase in dose-dependent growth response. - When the administration period of growth hormone was followed by a period without any hormone administration, the longitudinal bone growth was found to continue for some days after withdrawal, but then it ceased (paper V). A 10-day withdrawal period would be sufficient to register the full effect of different doses of growth hormone (paper V). This withdrawal effect can be used to increase the growth response in the bioassay of growth hormone.

Thyroxine administered in association with growth hormone resulted in a potentiating synergistic stimulation of the longitudinal bone growth (paper IV). At an optimum dose of thyroxine (20 µg/kg L-thyroxine) the dose of growth hormone was found to determine the increase in longitudinal bone growth. Thus, it is possible to determine the biological effect of various doses of growth hormone given in association with an optimum dose of thyroxine (paper IV).

The increase in the dose-dependent accumulated longitudinal bone growth by means of a withdrawal period and thyroxine administration allows determination of small doses of growth hormone during short periods. This was confirmed in papers XI and XII.

Modes of growth hormone administration. - Various factors of practical importance

for the bioassay of growth hormone, were investigated (paper VIII). The growth response was found to be almost the same for subcutaneous and intraperitoneal injection, whereas the intravenous injection route resulted in significantly lower response. The subcutaneous administration in various volumes did not significantly influence the dose-dependent growth response. Nor did freezing the dissolved growth hormone or adding NaOH to the solution.

Administration frequency. - When a total dose of growth hormone was divided into a different number of equal doses, given by subcutaneous injections distributed evenly over 10 days, the frequency of administration was found to have a significant influence on the dose-dependent longitudinal bone growth (paper X). An optimum growth stimulation was found for 1 inj./day in hypophysectomized rats and for 2 inj./day in thyroxine-treated hypophysectomized rats. Compared with the growth stimulating effect of this total growth hormone dose when given in one single injection, the growth response for the optimum administration frequency reached a 5-fold net increase in the hypophysectomized rats and a 9-fold net increase in the thyroxine-treated hypophysectomized rats. With a shorter administration period (5 days) in thyroxine-treated hypophysectomized rats, the growth stimulation induced by one daily growth hormone injection was the same as that induced by more frequent injections of the same total dose (paper X). Thus, it is possible to achieve an optimum growth response for a certain total dose of growth hormone by increasing the administration frequency. With the shorter administration period the longitudinal bone growth was optimum with one daily injection.

Stability of growth response. - When different doses of growth hormone were given - each dose to two different groups of animals at different occasions - no significant difference in the accumulated longitudinal bone growth was found between the groups given the same dose (paper VIII).

Also between experimental groups given the same growth hormone dose, but with other minor modifications of the experimental conditions, the corrected longitudinal bone growth response showed no significant difference, indicating a stability, whereas the uncorrected longitudinal bone growth showed minor differences. E.g., animals given low doses of cortisone (0.1 and 0.5 mg/kg) at hypophysectomy did not show any significant difference in corrected longitudinal bone growth compared to animals not given cortisone when the same doses of growth hormone were administered. This indicates both non-influence of the cortisone administration and stability of the growth response (paper VIII). Also a stability in the longitudinal bone growth response was indicated in the groups of animals given the same dose of growth hormone by different modes of administration (paper VIII). In the investigation with changes in the administration

frequency of growth hormone (paper X), no significant difference in the longitudinal bone growth was found with 10 days administration period when the optimum injection frequency was reached. With 5 days administration period, the dose-dependent longitudinal bone growth seemed very stable, showing no increase for administration frequency more than one daily injection (paper X). Furthermore, a linear log dose-response relation was found for dose-levels determined at different occasions (papers III, XI, XII).

Thus, the corrected longitudinal bone growth response for various doses of growth hormone has a great stability between different experimental occasions.

Specificity of growth response. - Growth hormone is prepared by extraction from pituitaries (see paper IX). Thus, contamination with other pituitary hormones might influence the growth hormone-induced longitudinal bone growth in a bioassay. It is then of value to know which pituitary hormones can stimulate longitudinal bone growth.

When the stimulating effect of different pituitary hormones on longitudinal bone growth was determined (paper IX), growth hormone was found to be the most effective. At considerably higher doses, thyroid-stimulating hormone (TSH) and prolactin showed growth stimulating activity. TSH was found to exert its effect via the production of thyroxine. The optimum dose of thyroxine used (20 µg/kg L-thyroxine) rendered the administration of TSH ineffective. The growth stimulation by prolactin seemed to be a direct effect of this hormone, similar to the effect of growth hormone. The other tested pituitary hormone preparations (LH, FSH, ACTH, MSH, vasopressin, and oxytocin) did not stimulate longitudinal bone growth.

The stimulating effects found for TSH and prolactin on longitudinal bone growth in the present study are mainly in accordance with earlier investigations into the influence of these hormones on width of the growth plate (see Geschwind and Li 1955, Papkoff and Li 1962).

A species specificity for growth hormone has been observed in man and many animals, but the rat grows when given growth hormone from many other species (Papkoff and Li 1962, Geschwind 1966, Hayashida 1972) and is thus suitable for the evaluation of various growth promoting substances. A substance that can stimulate growth in the rat might be a potential growth promoting agent also in other species.

3.7 Bioassay of growth hormone - different bioassay models (Papers III, XI, XII)

The longitudinal bone growth was found to increase with increased administration period when a constant daily dose of growth hormone was given (paper III). Both a short

administration period and a high response are desirable for the bioassay of growth hormone, but a shorter administration period results in a lower growth response (see papers III and V). To find a suitable bioassay model the effect of growth hormone on the longitudinal bone growth was investigated in four different models (papers XI and XII).

Growth hormone was given daily for 5 or 10 days, alone or in association with a daily injection of L-thyroxine, followed by a 10-day withdrawal period (papers XI and XII). A linear log dose-response relation with a high precision was found for all the models. Thus all the models could be used for the bioassay of growth hormone. All the dose-response-curves showed similar shapes with a plateau higher than the controls for the lowest growth hormone doses tested and a linearly increasing portion for the higher growth hormone doses. No levelling off in the dose-response effect for high doses was found with those used in the present investigation. Thyroxine-treatment increased the sensitivity of the bioassay. An administration period of 5 days proved most sufficient for the bioassay of growth hormone in thyroxine-treated hypophysectomized rats. This bioassay model was most favourable concerning sensitivity, precision, and length of administration period (paper XII).

Many investigations have been made to develop a more precise, specific, and sensitive bioassay method for growth hormone than those available. The earlier bioassay investigations have been extensively reviewed by Greenspan et al. (1950), Geschwind and Li (1955), Russel (1955), and Papkoff and Li (1962). Also more recent modifications of the bioassays based on increase in body weight (Goth et al. 1964, Parlow et al. 1965, Wallis and Dew 1973) and tail length (de Groot 1963), as well as modifications of the tibia test (Cargill Thompson and Crean 1963, Leget et al. 1969) have appeared (see also paper XII). Of the earlier bioassay methods for growth hormone, the tibia test based on increase in cartilage width in hypophysectomized rats (Evans et al. 1943, Greenspan et al. 1949) is the most satisfactory for sensitivity and injection period, and therefore most widely used.

The present bioassay method with a 5-day administration period in thyroxine-treated hypophysectomized rats has a satisfactory sensitivity and administration period, as in the tibia test. The lowest sensitivity for dose-response-determination was found to be a total dose of about 50 μ g growth hormone, whereas the sensitivity for a qualitative determination of growth stimulation (significantly different from controls but within the plateau) was a total dose of about 7 μ g. The precision ($\lambda = 0.148$ in paper XII and $\bar{\lambda} = 0.172$ in paper XIII) of the present bioassay method is better than it is in the ordinary tibia test, indicating a higher stability in the growth response. Also the longitudinal

bone growth is determined, which is the intended effect of clinical administration of growth hormone. In addition, the present bioassay method determines the total effect on the longitudinal bone growth of an administered dose of growth hormone, with the aid of a withdrawal period. This makes it possible to calculate the number of cells produced in the growth plate by the growth hormone dose, as already discussed (section 3.4).

Of the pituitary hormones, only growth hormone, TSH, and prolactin stimulated the growth in length in hypophysectomized rats (see paper IX). In the present bioassay, an optimum dose of thyroxine (paper IV) was used, which nullified the growth stimulation by TSH (paper IX). Thus, the present bioassay method with thyroxine treatment has the advantage of not being influenced by contamination in the growth hormone preparations by TSH, which is known to have stimulating effects on the tibia test (Geschwind and Li 1955, Papkoff and Li 1962). Thereby the specificity is increased in the present bioassay.

The advantages of the present bioassay would increase the possibility to determine the biological effect of growth stimulating substances. This was further investigated in paper XIII.

3.8 Bioassay of growth promoting substances - different growth hormone and prolactin preparations (Paper XIII)

The growth stimulating effect of different growth hormone and prolactin preparations on the longitudinal bone growth in thyroxine-treated hypophysectomized rats was determined. The hormones were administered for 5 days followed by a 10-day withdrawal period. The effect of the hormone preparations was compared with that of the 1:st International Standard for growth hormone (from WHO).

All the different tested growth hormones brought about a linear log dose-dependent stimulation of the longitudinal bone growth at a given interval. The corrected values for longitudinal bone growth resulted in lower standard deviation for the dose-response-lines than the uncorrected values. The potency calculation showed that the tested human growth hormone preparations had a higher potency than the bovine, ovine, and porcine growth hormone preparations. The human preparations had potencies between 3 and 5 IU/mg. The two bovine preparations differed in potency, containing about 2.4 IU/mg and 1.3 IU/mg. The ovine and one of the porcine growth hormone preparations had about 1 IU/mg; the other porcine preparation had lower potency (see paper XIII). Thus, potency differences were also found between hormones from the same species, but prepared by different methods or in different batches. When some of the bovine and human growth hormone

preparations were tested in repetitive experiments, and at different dose-levels, only minor differences in potency between the different determinations were found, indicating an almost constant growth response. When the dose-response-lines for the different preparations were compared with that of the WHO preparation, no significant departure from parallelism was found (see paper XIII).

The two different prolactin preparations resulted in a log dose-dependent stimulation of the longitudinal bone growth. They were found to have a considerably lower growth promoting activity (0.04 IU/mg) than the growth hormone preparations. The dose-response-line of the bovine prolactin preparation showed no significant departure from parallelism compared with that of the WHO growth hormone preparation, whereas the ovine prolactin preparation showed a divergence of low significance, probably owing to the comparatively low doses tested (see paper XIII).

The present bioassay method was used to determine the growth stimulating potency of various growth hormone and prolactin preparations from different species and prepared by different methods (paper XIII). Compared with earlier investigations (Collins et al. 1961, Damm et al. 1964, Goth and Arky 1968, Schleyer et al. 1970, Baetzner et al. 1972), where different growth hormone preparations have been studied, the present investigation (paper XIII) is most extensive, the growth stimulating effect of nine growth hormone and two prolactin preparations being determined. Differences in potency for the various hormones were found in the present study (paper XIII) compared with earlier bioassay determinations of these preparations. The earlier methods, however, used growth parameters other than the longitudinal bone growth; this could explain the differences. The present bioassay method seems to be more sensitive for the higher growth promoting potency (above 1.0 IU/mg) inherent in some preparations than the earlier methods used for testing these preparations (paper XIII). This might depend on a lower sensitivity in earlier methods for increasing doses of growth hormone. As shown previously (papers III, XI, XII), the body-weight-gain is considerably less suitable for the bioassay of growth hormone than the determination of longitudinal bone growth with tetracycline when determined in the same animals. Thus, it seems that the present bioassay method for growth stimulating substances, having a higher precision and specificity than earlier methods, increases the possibility of determining the biological effects of growth hormone preparations or parts of the growth hormone molecule thought to have growth stimulating activity.

Clinical substitution therapy with hormones given to hypopituitary patients presents problems concerning the possible interaction of different hormones with the growth hormone-induced longitudinal bone growth. The experimental model used in the present study would

make it possible to resolve the quantitative interactions between different growth stimulating and growth inhibiting substances.

Great international research is at present involved in preparing and modifying parts of the growth hormone protein molecule. In growth hormone from different species, a growth stimulating common amino acid sequence is sought. For testing these substances and also for the evolution of synthetic growth stimulating substances, a bioassay determining longitudinal bone growth is indispensable. According to Root (1972) the evolution of such substances is the field of future investigations concerning growth hormone.

In the present study, tetracycline was used as intravital marker to determine the longitudinal bone growth from the proximal growth plate of the tibia in the rat to obtain further and more exact quantitative information about the influence of growth hormone and other hormones on the process of growth in length of bones.

The longitudinal bone growth was determined in the normal and hypophysectomized rat. These investigations constitute a detailed base for the evaluation of further experimental influence.

It was possible to indicate the functional efficacy of a hypophysectomy by use of the determined values for postoperative accumulated longitudinal bone growth and width of a fluorescent band corresponding to a postoperative injection of tetracycline. This could be used as a criterion for the completeness of hypophysectomy.

Growth hormone was shown to stimulate the longitudinal bone growth in the hypophysectomized rat. The growth stimulation was dependent on the dose and the length of the administration period. Thyroxine was found to stimulate the longitudinal bone growth up to an optimum dose. Thyroxine and growth hormone given in association resulted in a potentiating synergistic stimulation of the growth in length.

The cell kinetics of the proximal tibial growth plate were calculated from the values of longitudinal bone growth and the size of the degenerative cell in the same animal. The changes in longitudinal bone growth with increasing age in normal rats, after hypophysectomy, and after subsequent administration of growth hormone and thyroxine, were found to be predominantly due to changes in the cell production and thereby the mitotic activity in the growth plate. Only minor changes were found in the cell size.

Growth hormone and thyroxine also influenced other growth parameters in the hypophysectomized rat, such as the width of the proximal tibial growth plate and the body and heart weights. The influence on these growth parameters, however, was quantitatively less pronounced than for the longitudinal bone growth.

A new bioassay method for growth promoting substances was developed, using as growth parameter the longitudinal bone growth determined with tetracycline in hypophysectomized rats.

Various factors important for determination of the dose-response effect in the bioassay of growth hormone were determined. The accumulated longitudinal bone growth was found to be better as growth parameter for bioassay than width of the growth plate and weight of body and heart, when determined in the same animals. The age and a high

dose of cortisone at hypophysectomy were found to influence the hormone-induced longitudinal bone growth. Different modes of administration of practical importance for the bioassay were investigated. Under standardized conditions, the dose-dependent growth response was found to be practically constant. The effect of administration frequency of growth hormone on the longitudinal bone growth was determined. It was possible to achieve an optimum growth response for a certain total dose of growth hormone given during a specific administration period by increasing the injection frequency.

Also the stimulating effect of different pituitary hormones on longitudinal bone growth was determined. Growth hormone was found to be most effective. At considerably higher doses also TSH (through the production of thyroxine) and prolactin showed growth stimulating activity. The LH, FSH, ACTH, MSH, vasopressin, and oxytocin preparations used did not stimulate the longitudinal bone growth.

When different models for the bioassay of growth hormone were tested, the model with 5 days administration period in thyroxine-treated hypophysectomized rats was found to be most favourable concerning sensitivity, precision, and specificity.

With this bioassay, the stimulating effects on the longitudinal bone growth of different growth hormone and prolactin preparations were compared with that of the 1st International Standard for growth hormone (WHO). The tested human growth hormone preparations had a higher potency than the bovine, ovine, and porcine growth hormone preparations. Also potency differences were found between hormones from the same species but prepared by different methods. As mentioned above, the prolactin preparations had a considerably lower growth promoting activity than the growth hormone preparations.

Compared with the earlier bioassay methods for growth hormone, the present bioassay is much more favourable, since a hormone-induced increase in bone length is determined directly. Furthermore, when all the factors, such as precision, sensitivity, specificity, and administration period are considered, the method is better than the previous ones. This method thus increases the possibility of determining the biological effects of various bone growth promoting substances, such as different growth hormone preparations, active parts of the growth hormone molecule, and synthetic substances with growth stimulating activity.

For facilities and scientific advice during this investigation sincere thanks go to: Professor Göran Bauer, professor Bengt Falck, and professor Gunnar Wiberg; docent Lars Ingvar Hansson, and docent Claus Rerup; docent Sven Arne Ahlgren, professor Dora Jacobsohn, docent Kerstin Menander-Huber, and Anders Stenström, M.D.

For technical assistance deep gratitude is due to:

The staff at the Departments of Histology and Orthopaedic Surgery, especially Mrs. Gunnel Sundin, Mrs. Birgitta Särgava, Mrs. Inga-Lill Bertilsson, and Mrs. Eva Ottosson.

This work was supported by the Faculty of Medicine, University of Lund, and by Alfred Österlund's Stiftelse, and was carried out within a research organisation sponsored by the Swedish Medical Research Council (Project 17X-2031).

- Ahlgren, S.A.: Rate of apposition of dentine in upper incisors in normal and hormone-treated rats. *Acta orthop.scand.*, Suppl. 116 (1968).
- Asling, C.W. and Evans, H.M.: Anterior pituitary regulation of skeletal development. In: *The Biochemistry and Physiology of Bone*, ed. Bourne, G.H., p. 671-703. New York: Academic Press (1956).
- Asling, C.W., Simpson, M.E. and Evans, H.M.: Gigantism: its induction by growth hormone in the skeleton of intact and hypophysectomized rats, and its failure following thyroidectomy. *Rev. suisse Zool.* 72, 1-37 (1965).
- Asling, C.W., Tse, F. and Rosenberg, L.L.: Effects of growth hormone and thyroxine on sequences of chondrogenesis in the epiphyseal cartilage plate. In: *Proceedings of the First International Symposium on Growth Hormone*, eds. Pecile, A. and Müller, E.E., p. 319-331. Amsterdam: Excerpta Medica (1968).
- Baetzner, P., Fehm, H.L., Voigt, K.H., Schleyer, M. and Pfeiffer, E.F.: Measurement of biological activity in various growth hormone preparations using a modified tibia test. *Acta endocr. (Kbh.)* 70, 231-238 (1972).
- Baume, L.J., Becks, H. and Evans, H.M.: Hormonal control of the caudal vertebrae in the rat. III. Skeletal response of hypophysectomized and normal female rats to hormonal therapy. *Bull. schweiz. Akad. med. Wiss.* 17, 231-245 (1958).
- Bliss, C.I.: *The Statistics of Bioassay*. New York: Academic Press (1952).
- Bliss, C.I. and Marks, H.P.: The biological assay of insulin. I. Some general considerations directed to increasing the precision of the curve relating dosage and graded response. *Quart. J. Pharm.* 12, 82-110 (1939).
- Cargill Thompson, H.E.C. and Crean, G.P.: Studies on the effect of hormone administration on body weight and on tibial epiphyseal cartilage width in intact, hypophysectomized and adrenalectomized rats. *J. Endocr.* 25, 473-482 (1963).
- Christensen, N.O.: Growth arrest by stapling. An experimental study of longitudinal bone growth and morphology of the growth region. *Acta orthop. scand.*, Suppl. 151 (1973).
- Collins, E.J., Lyster, S.C. and Carpenter, O.S.: Growth hormone and radiosulfate incorporation in costal cartilage. II. Comparison with the tibia test. *Acta endocr. (Kbh.)* 36, 51-56 (1961).

- Damm, H.C., Dominguez, J.M., Pensky, J. and Pearson, O.H.: Quantitative observations on the biological and immunological assays of human growth hormone. *Endocrinology* 74, 366-371 (1964).
- Dingemanse, E., Freud, J. and Uyldert, L.E.: Growth studies. I. In rats and in human dwarfs. *Acta endocr. (Kbh.)* 1, 71-96 (1948).
- Dixon, B.: Cartilage cell proliferation in the tail-vertebrae of new-born rats. *Cell Tiss. Kinet.* 4, 21-30 (1971).
- Evans, H.M., Briggs, J.H. and Dixon, J.S.: The physiology and chemistry of growth hormone. In: *The Pituitary Gland*, eds. Harris, G.W. and Donovan, B.T., Vol. 1, p. 439-491. London: Butterworths (1966).
- Evans, H.M. and Simpson, M.E.: Hormones of the anterior hypophysis. *Amer. J. Physiol.* 98, 511-546 (1931).
- Evans, H.M. Simpson, M.E., Marx, W. and Kibrick, E.: Bioassay of the pituitary growth hormone. Width of the proximal epiphyseal cartilage of the tibia in hypophysectomized rats. *Endocrinology* 32, 13-16 (1943).
- Evans, H.M., Uyei, N., Bartz, Q.R. and Simpson, M.E.: The purification of the anterior pituitary growth hormone by fractionation with ammonium sulfate. *Endocrinology* 22, 483-492 (1938).
- Fisher, R.A.: *Statistical Methods for Research Workers*. Edinburgh: Oliver and Boyd (1950).
- Fønss-Bech, P.: A study of growth hormone of anterior pituitary lobe. With special reference to determination of its biological potency. *Acta pharmacol. (Kbh.)*, Vol. 3, Suppl. 3 (1947).
- Geschwind, I.I.: Species specificity of anterior pituitary hormones. In: *The Pituitary Gland*, eds. Harris, G.W. and Donovan, B.T., Vol. 2, p. 589-612. London: Butterworths (1966).
- Geschwind, I.I. and Li, C.H.: The tibia test for growth hormone. In: *The Hypophyseal Growth Hormone, Nature and Actions*, eds. Smith, R.W., Gaebler, O.H. and Long, C.N.H., p. 28-58. New York, Toronto, London: Mc Graw-Hill Book Company Inc. (1955).
- Goth, E. and Arky, I.: Species specificity of somatotrophic hormone, the effect of human and bovine STH on the growth of rats and mice. *Acta physiol. Acad. Sci. hung.* 34, 25-28 (1968).

- Goth, A., Kis-Vigh, L. and Doby, A.: A simple method for the biological assay of human somatotrophic hormone. *Acta physiol. Acad. Sci. hung.* 25, 47-52 (1964).
- Greenspan, F.S., Li, C.H., Simpson, M.E. and Evans, H.M.: Bioassay of hypophyseal growth hormone: the tibia test. *Endocrinology* 45, 455-463 (1949).
- Greenspan, F.S., Li, C.H., Simpson, M.E. and Evans, H.M.: Growth hormone. In: *Hormone Assay*, ed. Emmens, C.W., p. 273-290. New York: Academic Press (1950).
- Groot, C.A. de: Tail growth in the thyroxine-treated hypophysectomized rat as a sensitive criterion for growth hormone activity. *Acta endocr. (Kbh.)* 42, 423-431 (1963).
- Ham, A.W.: Bone. In: *Histology 6:th ed.*, p. 388-460. Philadelphia and Toronto: Lippincott (1969).
- Hansson, L.I.: Determination of endochondral bone growth in rabbit by means of oxytetracycline. *Acta Univ. Lund. sectio II, No. 1* (1964).
- Hansson, L.I.: Daily growth in length of diaphysis measured by oxytetracycline in rabbit normally and after medullary plugging. *Acta orthop. scand., Suppl.* 101 (1967).
- Hansson, L.I., Stenström, A. and Thorngren, K.-G.: Skeletal deposition and toxicity of methacycline. *Nature (Lond.)* 219, 624-625 (1968).
- Hansson, L.I., Stenström, A. and Thorngren, K.-G.: Diurnal variation of longitudinal bone growth in rabbit. *Acta orthop. scand., in press* (1973).
- Hayashida, T.: Immunological reactions of pituitary hormones. In: *The Pituitary Gland*, eds. Harris, G.W. and Donovan, B.T., Vol. 2, p. 613-662. London: Butterworths (1966).
- Hayashida, T.: Comparative immunochemical studies of pituitary growth hormones. In: *Growth and Growth Hormone*, eds. Pecile, A. and Müller, E.E., p. 25-37. Amsterdam: Excerpta Medica (1972).
- Hedström, Ö.: Growth stimulation of long bones after fracture or similar trauma. A clinical and experimental study. *Acta orthop. scand., Suppl.* 122 (1969).
- Jacobsohn, D.: Effects of thyroxine on growth of mammary glands, whole body, heart and liver in hypophysectomized rats treated with insulin, cortisone and ovarian steroids. *Acta endocr. (Kbh.)* 35, 107-134 (1960).
- Jacobsohn, D.: The techniques and effects of hypophysectomy, pituitary stalk section and pituitary transplantation in experimental animals. In: *The Pituitary Gland*, eds. Harris, G.W. and Donovan, B.T., Vol. 2, p. 1-21. London: Butterworths (1966).

- Kember, N.F.: Cell division in endochondral ossification. A study of cell proliferation in rat bones by the method of tritiated thymidine autoradiography. *J. Bone Jt Surg. B-42*, 824-839 (1960).
- Kember, N.F.: Cell population kinetics of bone growth: The first ten years of autoradiographic studies with tritiated thymidine. *Clin. Orthop.* 76, 213-230 (1971 a).
- Kember, N.F.: Growth hormone and cartilage cell division in hypophysectomized rats. *Cell Tiss. Kinet.* 4, 193-199 (1971 b).
- Kember, N.F.: Comparative patterns of cell division in epiphyseal cartilage plates in the rat. *J. Anat. (Lond.)* 111, 137-142 (1972).
- Kember, N.F. and Walker, K.V.R.: Control of bone growth in rats. *Nature (Lond.)* 229, 428-429 (1971).
- Leget, J.N., Reitsma, J.W., Louwerens, B. and Kouwenberg, J.: A rapid growth hormone test by radiography. *Int. J. Protein Res.* 1, 81-83 (1969).
- Loraine, J.A. and Bell, E.T.: Growth hormone. In: *Hormone Assays and their Clinical Application*, eds. Loraine, J.A. and Bell, E.T., p. 150-166. Edinburgh and London: Livingstone (1966).
- Marx, W., Simpson, M.E. and Evans, H.M.: Bioassay of the growth hormone of the anterior pituitary. *Endocrinology* 30, 1-10 (1942).
- Marx, W., Simpson, M.E., Li, C.H. and Evans, H.M.: Antagonism of pituitary adrenocorticotrophic hormone to growth hormone in hypophysectomized rats. *Endocrinology* 33, 102-105 (1943).
- McLean, F.C. and Urist, M.R.: *Bone. Fundamentals of the Physiology of Skeletal Tissue*, 3:d ed.. Chicago and London: The University of Chicago Press (1968).
- Müller, E.E., Giustina, G., Miedico, D., Cocchi, D. and Pecile, A.: Analogous pattern of bioassayable and radioimmunoassayable growth hormone in some experimental conditions of the rat and mouse. In: *Growth and Growth Hormone*, eds. Pecile, A. and Müller, E.E., p. 283-298. Amsterdam: Excerpta Medica (1972).
- Papkoff, H. and Li, C.H.: Hypophyseal growth hormone. In: *Methods in Hormone Research, Vol. II Bioassay*, ed. Dorfmann, I., p. 671-704. New York: Academic Press (1962).
- Parlow, A.F., Wilhelmi, A.E. and Reichert, L.E.: Further studies on the fractionation of human pituitary glands. *Endocrinology* 77, 1126-1134 (1965).
- Persson, B.M.: Growth in length of bones in change of oxygen and carbon dioxide tensions. *Acta orthop. scand., Suppl.* 117 (1968).
- Rang, M.: *The growth plate and its disorders*. Edinburgh and London: Livingstone (1969).

- Riekstniece, E. and Asling, C.W.: Thyroxine augmentation of growth hormone-induced endochondral osteogenesis. *Proc. Soc. exp. Biol. (N.Y.)* 123, 258-263 (1966).
- Rigal, W.M.: The use of tritiated thymidine in studies of chondrogenesis. In: *Radioisotopes and Bone*, eds. McLean, F.C., Lacroix, P. and Budy, A.M., p. 197-219. Oxford: Blackwell Scientific Publications (1962).
- Root, A.W.: *Human Pituitary Growth Hormone*. Springfield: C.C. Thomas (1972).
- Russel, J.A.: Methods of detection and assay of growth hormone. In: *The Hypophyseal Growth Hormone, Nature and Actions*, eds. Smith, R.W., Gaebler, O.H. and Long, C.N.H., p. 17-27. New York, Toronto, London: Mc Graw-Hill Book Company Inc. (1955).
- Schleyer, M., Schröder, K.E. and Hinz, A.: Comparative investigations of different human growth hormone preparations. *Horm. Metab. Res.* 2, 174-180 (1970).
- Scow, R.O.: Effect of thyroxine on the weight and composition of muscle, pelt and other tissues in young hypophysectomized rats. *Endocrinology* 55, 344-353 (1954).
- Simpson, M.E., Asling, C.W. and Evans, H.M.: Some endocrine influences on skeletal growth and differentiation. *Yale J. Biol. Med.* 23, 1-27 (1950).
- Sissons, H.A.: Experimental study of the effect of local irradiation on bone growth. In: *Progress in Radiobiology, Proc. Fourth Intern. Conf. Radiobiology*, eds. Mitchell, J.S., Holmes, B.E. and Smith, C.L., p. 436-448. Edinburgh and London: Oliver and Boyd (1955).
- Sissons, H.A.: The growth of bone. In: *The Biochemistry and Physiology of Bone*, ed. Bourne, G.H., p. 443-474. New York: Academic Press (1956).
- Sissons, H.A.: The growth of bone. In: *The Biochemistry and Physiology of Bone, Vol. III Development and Growth*, ed. Bourne, G.H., p. 145-180. New York and London: Academic Press (1971).
- Smith, P.E.: Hypophysectomy and a replacement therapy in the rat. *Amer. J. Anat.* 45, 205-256 (1930).
- Soyka, L.F. and Crawford, J.D.: Antagonism by cortisone of the linear growth induced in hypopituitary patients and hypophysectomized rats by human growth hormone. *J. clin. Endocr.* 25, 469-475 (1965).
- Sundén, G.: Some aspects of longitudinal bone growth. An experimental study of the rabbit tibia. *Acta orthop. scand.*, Suppl. 103 (1967).
- Urist, M.R.: Growth hormone and skeletal tissue metabolism. In: *The Biochemistry and Physiology of Bone, Vol. II Physiology and Pathology*, ed. Bourne, G.H., p. 155-194. New York and London: Academic Press (1972).

- Walker, K.V.R. and Kember, N.F.: Cell kinetics of growth cartilage in the rat tibia.
I. Measurements in young male rats. *Cell Tiss. Kinet.* 5, 401-408 (1972 a).
- Walker, K.V.R. and Kember, N.F.: Cell kinetics of growth cartilage in the rat tibia.
II. Measurements during ageing. *Cell Tiss. Kinet.* 5, 409-419 (1972 b).
- Wallis, M. and Dew, J.A.: The bioassay of growth hormone in Snell's dwarf mice:
Effects of thyroxine and prolactin on the dose-response curve. *J. Endocr.* 56,
235-243 (1973).

